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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/00, 31/34, 31/35

A1

(11) International Publication Number: WO 97/41844

(43) International Publication Date: 13 November 1997 (13.11.97)

(21) International Application Number: PCT/US97/05574

(22) International Filing Date: 3 April 1997 (03.04.97)

(30) Priority Data:
60/017,096 9 May 1996 (09.05.96) US

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: COMBINATIONS OF ANGIOSTATIC COMPOUNDS

(57) Abstract

The present invention is directed to compositions containing combinations of angiostatic compounds and methods for their use in preventing pathological neovascularization.

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COMBINATIONS OF ANGIOSTATIC COMPOUNDS

Background of the invention

The present invention relates to certain compounds useful in preventing and treating neovascularization. Specifically, the invention is directed to compositions containing two or more combinations of angiostatic agents and methods of using combinations of these angiostatic agents to treat neovascularization.

Angiogenesis is a term used to describe the development of new blood vessels or neovascularization (L. Diaz-Flores et al., *Angiogenesis: an Update*, Histology and Histopathology, volume 9, pages 807-843 (1994)). Though angiogenesis is a normal process for the development or maintenance of the vasculature, pathological conditions (i.e., angiogenesis dependent diseases) arise where blood vessel growth is actually harmful. Such pathologies include diabetic retinopathies, proliferative vitreoretinopathies, psoriasis, arthritis and solid tumor development. The progression of angiogenesis occurs in several phases which include: elaboration of the angiogenic signal; dissolution of the blood vessel basement membrane; endothelial cell proliferation; endothelial cell migration; and formation and differentiation of capillary tubules and loops. Each of these phases is a potential target for pharmacological intervention.

Tumor growth is dependent on neovascularization. For solid tumors to grow beyond the size of a pea, they must become vascularized. They do so by secreting their

own angiogenic factor(s) which recruit new blood vessels to provide essential nutrients and oxygen.

Angiogenesis is also associated with important diseases of ocular tissue especially in older patients and diabetics. Any abnormal growth of blood vessels in the eye can scatter and block the incident light prior to reaching the retina. Neovascularization can occur at almost any site in the eye and significantly alter ocular tissue function. Some of the most threatening ocular neovascular diseases are those which involve the retina. For example, many diabetic patients develop a retinopathy which is characterized by the formation of leaky, new blood vessels on the anterior surface of the retina and in the vitreous causing proliferative vitreoretinopathy. A subset of patients with age related macular degeneration develop subretinal neovascularization which leads to their eventual blindness.

Current therapy for the treatment of ocular neovascular disease is not very effective. Retinal neovascularization is often treated with multiple laser burns to the retina to remove the pathological vasculature. Panretinal photocoagulation, however, destroys normal retinal tissue. Patients with neovascular diseases of the anterior chamber (e.g. corneal neovascularization, iritis rubeosis) are treated with potent topical ocular glucocorticoids. These therapies are only partially effective and generally only slow neovascularization and the progress of the overall disease. In addition, they can cause severe side effects if used over a relatively long period of time.

Other attempts have been made to provide therapies for the prevention or treatment of pathological angiogenesis. For example, angiostatic steroids functioning to inhibit angiogenesis in the presence of heparin or specific heparin fragments in the

chicken embryo model of neovascularization are disclosed in Crum, et al., *A New Class of Steroids Inhibits Angiogenesis in the Presence of Heparin or a Heparin Fragment*, Science, volume 230, pages 1375-1378 (1985). Other groups of angiostatic steroids useful in inhibiting angiogenesis are disclosed in commonly assigned WIPO Publication No. WO 93/10141 (Clark et al.) and United States Patent No. 5,371,078 (Clark et al.), as well as WO 95/18621 (Prioa et al.).

Glucocorticoids, as mentioned above, have also been shown to inhibit angiogenesis. However, the use of glucocorticoid therapy in general is complicated by the inherent problems associated with steroid applications. Such problems include elevated intraocular pressure (Kitazawa, *Increased Intraocular Pressure Induced by Corticosteroids*, American Journal of Ophthalmology, volume 82, pages 492-495 (1976)), and the development of posterior subcapsular cataracts.

Suramin, a complex molecule, has been described as a growth factor antagonist and possessing angiosuppressive action (Takano, *Angiosuppressive and Antiproliferative Actions of Suramin: A Growth Factor Antagonist*, Growth Factors, Peptides and Receptors, Ed. T.W. Moody, Plenum Press, New York, pages 255-264 (1993)). Suramin and its derivatives have also been disclosed in WIPO Publication No. WO 90/15816, as inhibitors of fibroblast growth factor (an angiogenesis factor) as well as apparent angiostatic agents.

Fumagillin and analogs of fumagillin have been reported to possess angiostatic properties (Ingber et al., *Synthetic Analogues of Fumagillin that Inhibit Angiogenesis and Suppress Tumor Growth*, Nature, volume 348, pages 555-557 (1990)). Several European patent applications have disclosed fumagillin analogs including: European Patent

Application Nos. 0 354 787 A1, 0 386 667 A1 and 0 470 569 A1, all assigned to Takeda Chemical (Japan) or Fujisawa Pharmaceutical (Japan).

Anti-estrogens, or estrogen antagonists, have also been reported to possess angiostatic activity. Antiestrogens have been shown to alter the activity of a number of growth factors that are important in the control of cellular proliferation (Freiss et al. *Antisteroidal and anti-growth factor activities of antiestrogens*, Journal of Steroid Biochemistry and Molecular Biology, volume 37, pages 777-781 (1990)). They are known to affect biochemical functions and to inhibit angiogenesis in a dose dependent manner (Gagliardi et al., *Inhibition of Angiogenesis by Antiestrogens*, Cancer Research, volume 53, pages 533-535 (1993)).

Still other therapies have been proposed, including, the use of protamine (S. Taylor, *Protamine is an Inhibitor of Angiogenesis*, Nature, volume 297, pages 307-312 (1982)), and the use of Vitamin D_3 analogs (Oikawa et al., *Inhibition of Angiogenesis by Vitamin D_3 Analogues*, European Journal of Pharmacology, volume 178, pages 247-250 (1990)). The use of a variety of pharmaceutical proteins has also been proposed for treating angiogenesis. Such therapies have included: monoclonal antibodies directed to fibroblast growth factor, disclosed in WO 91/06668; platelet factor 4, disclosed in WO 93/02192; and thrombospondin fragments, disclosed in WO 93/16716.

Summary of the Invention

The present invention involves the angiostatic therapy of a combination of two or more molecules selected from a set of angiostatic compounds. As such, the present

invention is directed to methods of using combinations of angiostatic compounds for the prevention and/or treatment of neovascularization in human patients. The present invention is also directed to compositions containing combinations of angiostatic compounds. In particular, the compositions are useful for controlling ocular neovascularization.

The combination therapy of the present invention is believed to have the advantage of providing effective, multi-mechanistic angiostatic therapy which is more efficacious with fewer side effects.

Detailed Description of the Invention

The compositions and methods of the present invention involve various combinations of angiostatic compounds. Table 1 contains a list of different angiostatic compounds useful in the present invention:

Table 1

Classes of Angiostatic Agents (examples where specified)

Anti-mitotics (5-flurouracil, mytomycin-C, taxol)
Estrogen metabolites (2-methoxyestradiol)
Matrix metalloproteinase inhibitors (betimastat, BB-2516, TIMPs, minocycline, GM6001)
Plasminogen activator/urokinase inhibitors
Urokinase receptor antagonists
Platelet factor 4 and analogs

Heparinases

Cartilage-derived inhibitor of angiogenesis

Thrombospondin and related analogs (TSP-1)

Angiostatin, vasculostatin

Proliferin-related protein

Fumagillin analogs (TNP-470)

Tecogalan

Pentosan polysulfate

Thalidomide and related analogs

CM101

Tyrosine kinase inhibitors (SU101)

Anti-sense oligonucleotides (specific for bFGF and VEGF)

Suramin and suramin analogs

Angiostatic steriods

$\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonists

Cytotoxic antibodies against endothelial cell antigens (anti-endoglin)

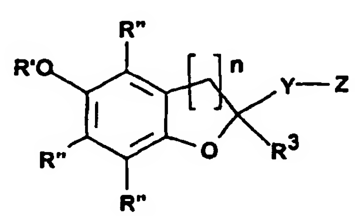
Interferon (α-IFN)

VEGF and bFGF antagonists (VEGF receptor-chimeric proteins)

flk-1 and flt-1 antagonists

IL-1, and TNF antagonists

Additionally, compounds of formula (I) below are also useful angiostatic compounds of the present invention:



(I)

wherein:

n is 1 or 2;

R is H, C_1-C_6 alkyl or C_3-C_6 cycloalkyl;

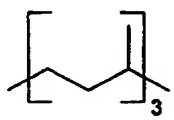
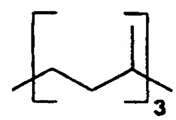
Y is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, O, NR, $C(R)_2$, CH(OH) or $S(O)_{n'}$;

n' is 0 to 2;

R' is H, C(O)R, $C(O)N(R)_2$, PO_3^-, SO_3^-, or $HO_2C(CH_2)_2(C{=}O)$---;

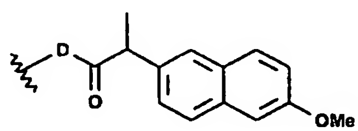
R" is H or C_1-C_6 alkyl;

R^3 is H, C_1-C_6 alkyl, $(CH_2)_q(OH)$, ---$(C{=}O)O(CH_2)_qCH_3$ or

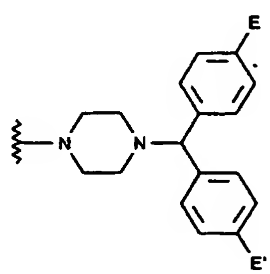
 ; provided that R' can not be H, when R^3 is  ;

q is 1 to 10; and

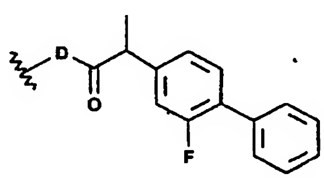
Z, if present, is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, or selected from the group consisting of:



;



; and



;

wherein:

D is O or NR; and

E and E' are independently H, F or Cl.

The compounds of the present invention also include pharmaceutically acceptable salts of the compounds of formula (I) and the compounds included in the angiostatic classes listed on Table 1.

The initiation of new blood vessel formation may arise quite differently in various tissues or as a result of different diseases. Many substances have been found to induce neovascularization; see, Folkman, et al., *Angiogenic Factors*, Science, volume 235, pages 442-447 (1987). It is believed, however, that once initiated, the process of neovascularization is similar in all tissues regardless of the associated disease; see, Furcht, *Critical Factors Controlling Angiogenesis: Cell Products, Cell Matrix, and Growth Factors*, Laboratory Investigation, volume 55, No. 5, pages 505-509 (1986).

There are many theories associated with the cause of neovascularization, and there may be different inducers depending on the disease or surgery involved; see, BenEzra, *Neovasculogenic Ability of Prostaglandins, Growth Factors, and Synthetic Chemoattractants*, American Journal of Ophthalmology, volume 86, No. 4, pages 455-461, (October, 1978). Regardless of the cause or the associated disease, it is believed that angiostatic agents work by inhibiting one or more steps in the process of neovascularization.

As stated above, angiogenesis is brought about through a number of biochemical and cellular mechanisms involving several steps, each of which is a potential target for pharmacological intervention. This process generally consists of the following steps: breakdown of blood vessel basement membranes, endothelial cell activation, migration, proliferation and formation of capillary tubules. In response to an angiogenic signal, endothelial cells become "activated" and release proteases and other degradative enzymes

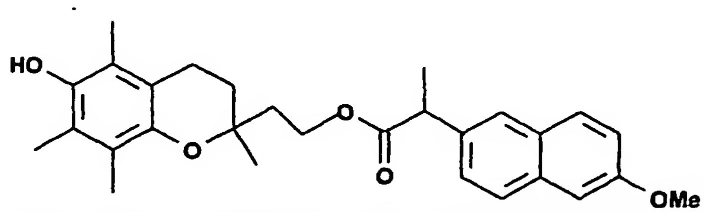
which dissolve the basement membranes surrounding the cells. The endothelial cells can now migrate towards the stimulus elongating and aligning themselves to create a sprout. Rapid proliferation lengthens the columns of cells and branches will appear at the top of the column. Branches from adjacent columns fuse and in this way a network of new capillaries is formed. Thus, inhibition of this complex process of angiogenesis is possible at several stages. These include interception of the activation signal, inhibition of basement membrane breakdown, inhibition of cell migration, inhibition of cell proliferation as well as interference with the formation of capillary tubules.

Different compounds may affect different stages of the angiogenic process. For example, antibodies to growth factors such as bFGF (Hori et al., *Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against human basic fibroblast growth factor*, Cancer Research, volume 51, pages 6180-6184 (1991)) and VEGF (Kim et al., *Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo*, Nature, volume 362, pages 841-844 (1993)) can interfere with the activation signal; fumagillin-type and suramin-type compounds inhibit endothelial cell migration; chroman derivatives and fumagillin-type compounds (Ingber et al., *Synthetic analogs of fumagillin that inhibit angiogenesis and suppress tumour growth*, Nature, volume 348, pages 555-557 (1990)) and suramin-type compounds (C. Stein, *Suramin: A novel antineoplastic agent with multiple potential mechanisms of action*, Cancer Research, volume 53, pages 2239-2248 (1993)) inhibit cell proliferation; and angiostatic steroids are currently thought to exert their anti-angiogenic effects by inhibiting basement membrane breakdown (Ashino-Fuse et al., *Medroxyprogesterone Acetate, An Anti-Cancer And Anti-Angiogenic Steroid, Inhibits The Plasminogen*

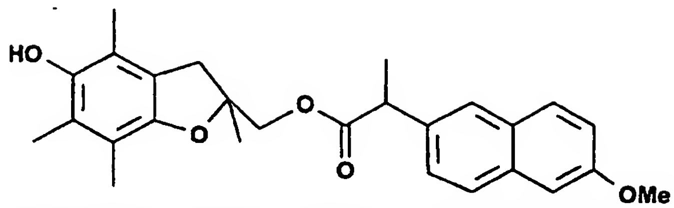
Activator In Bovine Endothelial Cells, volume 44, pages 859-864 (1989)) or by inhibiting PAI-1 synthesis (Blei et al., *Mechanism of Action of Angiostatic Steriods: Suppression of Plasminogen Activator Activity Via Stimulation of Plasminogen Activator Inhibitor Synthesis*, Journal of Cellular Physiology, volume 155, pages 568-578 (1993)). Therefore, therapeutic intervention is possible at several points in the process.

While applicants do not wish to be bound by any theory, it is believed that the inhibition of multiple cellular/biological mechanisms associated with angiogenesis will more effectively inhibit neovascularization. Therefore, the use of combinations of compounds affecting different mechanisms of angiogenesis would be more effective in preventing neovascularization than a single therapeutic approach. For example, combinations of anti-proliferative compounds such as chroman derivatives and angiostatic steroids which affect basement membrane breakdown would be more effective in preventing neovascularization than either drug alone. Therefore, the present invention sets forth the use of combinations of different angiostatic agents to provide a more effective therapeutic approach to inhibiting neovascularization. As used herein, the term "angiostatic agent or compound" refers to any compound which inhibits one or more processes of angiogenesis such that angiogenesis is inhibited or retarded.

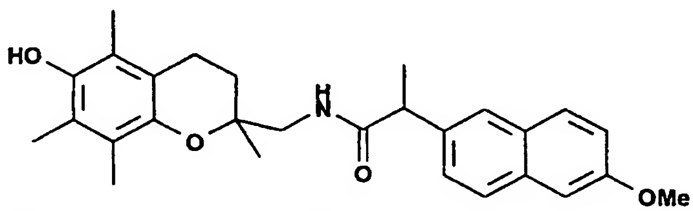
Preferred compounds of formula (I), also known as "chroman derivatives," include the following:



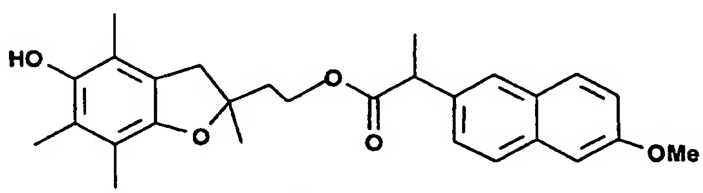
2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2-yl)ethyl 2-(6-methoxy-2-naphthyl)propionate ("Compound A");



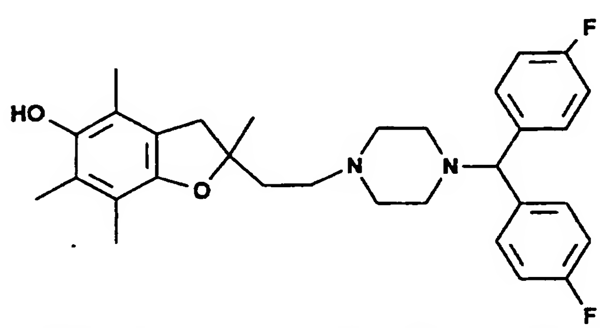
2-(5-hydroxy-2,4,6,7-tetramethyl-2,3-dihydro-benzo[1,2-b]furan-2-yl)methyl 2-(6-methoxy-2-naphthyl)propionate ("Compound B");



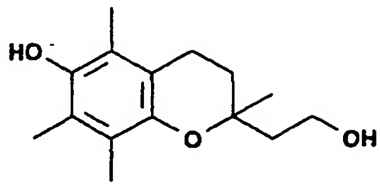
N-(2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2-yl)methyl) 2-(6-methoxy-2-naphthyl)propionamide ("Compound C");



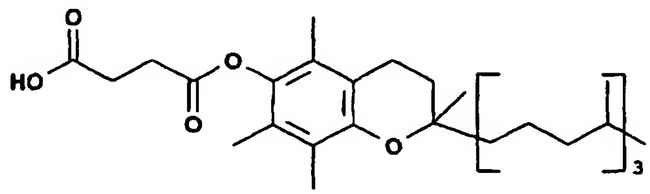
2-(5-hydroxy-2,4,6,7-tetramethyl-2,3-dihydro-benzo[1,2-b]furan-2-yl)ethyl 2-(6-methoxy-2-naphthyl)propionate ("Compound D");



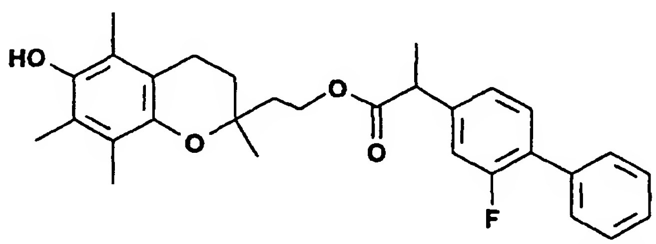
1-[2-(5-hydroxy-2,4,6,7-tetramethyl-2,3-dihydro-benzo[1,2-b]furan-2-yl)2-ethyl]-4-[4,4'-fluorobenzhydryl]piperzine ("Compound E")



2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2-yl)ethanol ("Compound F")



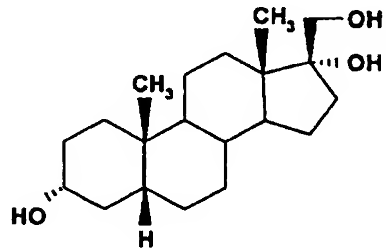
Vitamin E succinate (VES) ("Compound G")



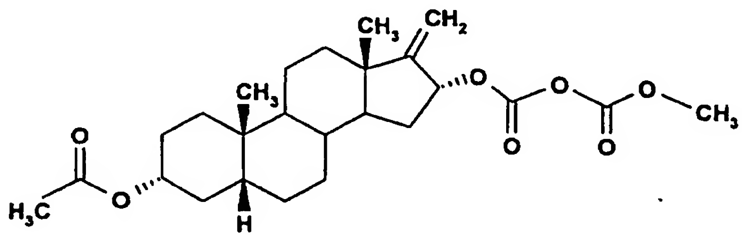
2-(6-hydroxy-2,5,7,8-tetramethyl-2,3-dihydro-2H-benzo[1,2-b]pyran-2-yl)ethyl 2-(3-fluoro-4-phenyl-phenyl)propionate ("Compound H").

"Angiostatic steroids" are compounds containing the 6-6-6-5-ring steroid backbone and possessing angiostatic activity. Preferred angiostatic steroids of the present invention have been disclosed in United States Patent No. 5,371,078 (Clark et al.) and WIPO Publication WO 93/10141 (Clark et al.); the entire contents of these publications are incorporated herein by reference. Preferred angiostatic steriods are:

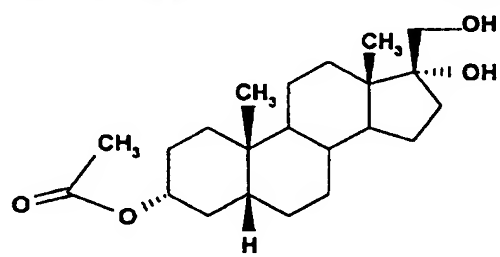
21-Nor-5ß-pregnan-3α,17α,20-triol



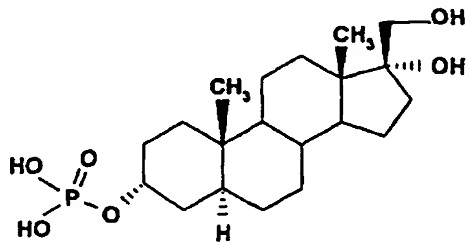
21-Nor-5ß-pregn-17(20)en-3α,16-diol-3-acetate-16-(O-methyl)malonate



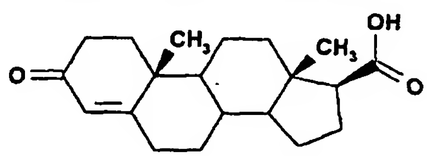
21-Nor-5ß-pregnan-3α,17α,20-triol-3-acetate



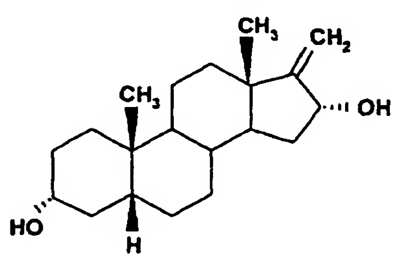
21-Nor-5α-pregnan-3α,17α,20-triol-3-phosphate



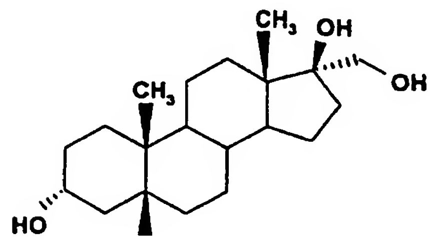
4-Androsten-3-one-17ß-carboxylic acid



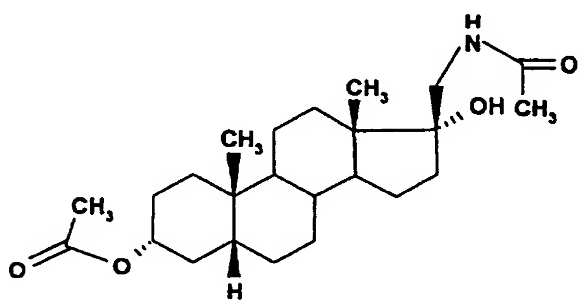
21-Nor-5ß-pregn-17(20)en-3α,16-diol



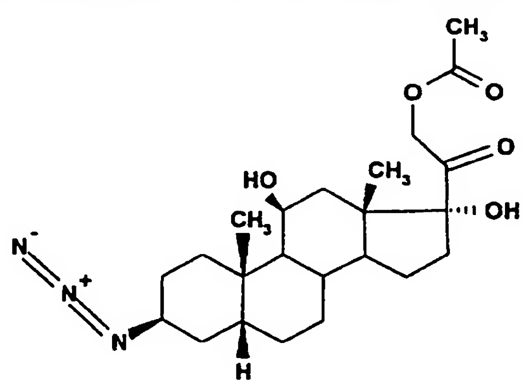
21-Nor-5ß-pregnan-3α,17 ß,20-triol



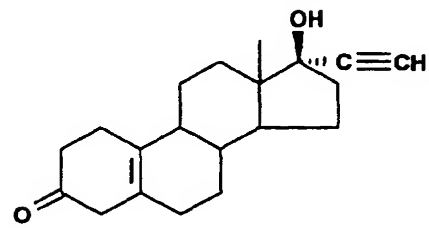
20-Acetamido-21-nor-5ß-pregnan-3α,17α-diol-3-acetate



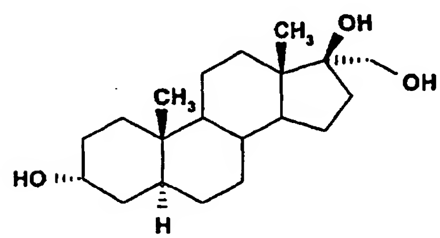
3ß-Azido-5ß-pregnan-11 ß,17α,21-triol-20-one-21-acetate



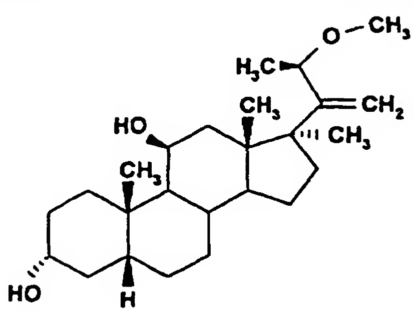
17α-Ethynyl-5(10)-estren-17ß-ol-3-one



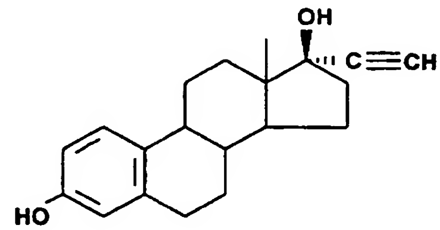
21-Nor-5α-pregnan-3α,17ß,20-triol



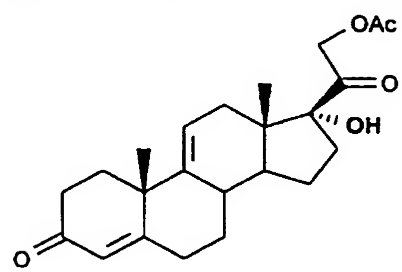
21α-Methyl-5ß-pregnan-3α,11ß,17α,21-tetrol-20-one-21-methyl ether



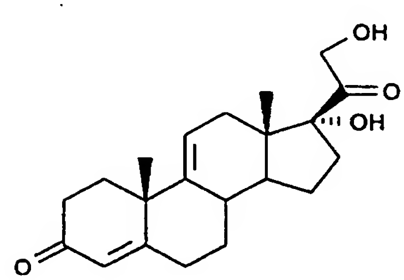
17α-Ethynyl-1,3,5(10)-estratrien-3,17ß-diol



4,9(11)-Pregnadien-17α,21-diol-3,20-dione-21-acetate



4,9(11)-Pregnadien-17α,21-diol-3,20-dione



"Suramin-type compounds" are compounds which mimic the anti-angiogenic action of suramin. Suramin and suramin-type compounds are known to those skilled in the art. Examples of suramin-type compounds are:

suramin,

3-hydroxy-2,7-naphthalenesulfonic acid,

4,5-dihydroxy-2, 7-naphthalenedisulfonic acid,

2,2′-[(1,8-dihydroxy-3,6-disulfo-2,7-napthylene)bis(azo]di-benzenearsonic acid,

4,4′-bis[[4-(o-hydroxyanilino)-6-(m-sulfoanilino)-s-triazin-2-yl]amino]-2,2′stilbenedisulfonic acid,

4,5-dihydroxy-3-[(p-nitrophenyl)azo]-2,7-naphthalenedisulfonic acid,

4,5-dihydroxy-3,6-bis[(4-sulfo-1-naphthyl)azo]-2,7-naphthalene-disulfonic acid,

3-[(5-chloro-2-hydroxyphenyl)azo]-4,5-dihydroxy-2,7-naphthalene-disulfonic acid,

4,5′-dihydroxy-3,6′[3,3′-dimethoxy-4,4′-biphenylylene)bis(azo)-di-1-naphthalenesulfonic acid,

3,6-[(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)azo]-4,5-dihydroxy-2,7-naphthalenedisulfonic acid,

5,5′-[ureylenebis[2-sulfo-p-phenylene_azo]bis[6-amino-4-hydroxy-2-naphthalenesulfonic acid,

4-[(o-arsonophenyl)azo]3-hydroxy-2,7-naphthalenedisulfonic acid,

4,5-dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid,

4-acetamido-5-hydroxy-6-(phenylazo)-1,7-naphthalenedisulfonic acid,

2-[p-[(1-hydroxy-4-sulfo-2-naphthyl)azo]phenyl]-6-methyl-7-benzothiazolesulfonic acid,

4-[(2,4-dimethylphenyl)azo]-3-hydroxy-2,7-napthalenedisulfonic acid,

3-[(4-Sulfophenyl)azo]-4,5-dihydroxy-2,7-naphthalenedisulfonic acid,

3-[(4-nitrophenyl)azo]-4-amino-5-hydroxy-2,7-naphthalene-disulfonic acid,

1-nitro-4,6,8-naphthalenetrisulfonic acid,

1-amino-4,6,8-naphthalenetrisulfonic acid and pharmaceutically acceptable salts thereof.

The most preferred suramin-type compounds include: suramin and 4,4′-bis[[4-(o-hydroxyanilino)-6-(m-sulfoanilino)-s-triazin-2-yl]amino]-2,2′stilbenedisulfonic acid.

"Fumagillin-type compounds" are oxaspiro[2,5]octane derivatives, such as those described in European Patent Application Nos. 0 354 787 A1, 0 386 667 A1 and 0 387

650 A1, the entire contents of these three publications are incorporated herein by reference to the extent they disclose angiostatic fumagillin-type compounds.

"Anti-estrogen compounds" are those compounds which at least partially bind estrogen receptors. A number of anti-estrogens have been shown to inhibit angiogenesis. Anti-estrogen compounds of the present invention include: clomiphene, tamoxifen, nafoxidine, ICI 164,984 and ICI 182,780.

Other angiostatic compounds contained in the Table 1 are known in the art to possess angiostatic activity. Of those classes listed, 5-flurouracil, mitomycin-C, taxol, 2-methoxyestradiol, betimastat, BB-2516, TIMPs, minocycline, GM6001, PF4, CDI, TSP-1, TNP-470, SU101, anti-endoglin, α-IFN and VEGF receptor-chimeric proteins are preferred for use in combinations of angiostatic compounds of the present invention.

The following are the most preferred types of angiostatic compounds to be included in combinations of angiostatic compounds of the present invention:

compounds of formula (I), anti-mitotics, angiostatic steroids, fumagillin-type compounds, suramin-type compounds, estrogen metabolites, matrix metalloproteinase inhibitors and thalidomide.

The following are the most preferred combinations of compounds to be used in compositions and methods of the present invention:

1) A compound of formula (I) and an angiostatic steroid
2) A compound of formula (I) and a suramin-type compound
3) A compound of formula (I) and a fumagillin-type compound
4) A compound of formula (I) and an anti-mitotic
5) An angiostatic steroid and a fumagillin-type compound
6) An anti-mitotic and an angiostatic steriod
7) A compound of formula (I) and an estrogen derivative
8) An angiostatic steroid and an estrogen metabolite
9) A compound of formula (I) and a matrix metalloprotease inhibitor

The combination of angiostatic compounds of the present invention are useful in inhibiting pathological neovascularization in human patients. As used herein, the term

"pathological neovascularization" refers to those conditions where the formation of blood vessels (neovascularization) is harmful to the patient. Examples of pathological neovascularization dependent diseases include: head trauma, spinal trauma, systemic or traumatic shock, stroke, hemorrhagic shock, cancer, arthritis, arteriosclerosis, angiofibroma, arteriovenous malformations, corneal graft neovascularization, inappropriate wound healing, diabetic retinopathy, granulations, burns, hemangioma, hemophilic joints, hypertrophic scars, ocular neovascularization, nonunion fractures, Osler-Weber Syndrome, psoriasis, pyogenic granuloma, retrolental fibroplasia, scleroderma, trachoma, vascular adhesions, and solid tumor growth.

In particular, the compositions of the present invention are useful in preventing and treating any ocular neovascularization, including, but not limited to: retinal diseases (diabetic retinopathy, chronic glaucoma, retinal detachment, sickle cell retinopathy and subretinal neovascularization due to age related macular degeneration); rubeosis iritis; proliferative vitreoretinopathy; inflammatory diseases; chronic uveitis; neoplasms (retinoblastoma, pseudoglioma and melanoma); Fuchs' heterochromic iridocyclitis; neovascular glaucoma; corneal neovascularization (inflammatory, transplantation and developmental hypoplasia of the iris); neovascularization following a combined vitrectomy and lensectomy; vascular diseases (retinal ischemia, choroidal vascular insufficiency, choroidal thrombosis and carotid artery ischemia); neovascularization of the optic nerve; and neovascularization due to penetration of the eye or contusive ocular injury.

Additionally the combinations of angiostatic agents are useful in treating pterygium (primary and recurrent), glaucoma filtration surgery bleb failure, hyperkeratosis, cheloid formation and polyp formation.

The use of the compositions of the present invention to ameliorate complications arising from glaucoma filtration surgery is a particularly important aspect of the invention. Glaucoma filtration surgery involves the surgical creation of a fistula with a conjuctival flap which allows the direct drainage of aqueous humor from the anterior chamber into the conjuctival tissue thereby lowering the elevated intraocular pressure associated with glaucoma. However, in many patients, the filtration "bleb" becomes scarred or healed over so that aqueous drainage can no longer occur. It has been noted that failing filtration blebs may become vascularized prior to failure. This vascularization may feed the fibroblasts which migrate, and proliferate, and block the bleb, or the vascularization itself may also result in physical blockage of the bleb. It is therefore likely that inhibition of filtration bleb neovascularization may inhibit filtration bleb failure.

The angiostatic compounds may be contained in various types of pharmaceutical compositions, either together as a single composition or in separate compositions, in accordance with formulation techniques known to those skilled in the art. For example, the compounds may be included in tablets, capsules, solutions, suspensions and other dosage forms adapted for oral administration; solutions and suspensions adapted for parenteral use; solutions, suspensions or gels for topical ocular administration; solutions and suspensions adapted for intra-vitreal or intra-cameral use; and suppositories for rectal use. Solutions, suspensions and other dosage forms adapted for topical application to the

involved tissues, such as tissue irrigating solutions, are particularly preferred for treatment of acute conditions associated with surgery or other forms of trauma.

The present invention is particularly directed to the provision of compositions adapted for treatment of ophthalmic tissues. Various types of vehicles may be used. The vehicles will generally be aqueous in nature. Aqueous solutions are generally preferred, based on ease of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the compounds of the present invention may also be readily incorporated into other types of compositions, such as suspensions, viscous or semi-viscous gels or other types of solid or semi-solid compositions. Suspensions may be preferred for compounds of the present invention which are relatively insoluble in water. The ophthalmic compositions of the present invention may also include various other ingredients, such as buffers, preservatives, co-solvents and viscosity building agents.

An appropriate buffer system (e.g., sodium phosphate, sodium acetate or sodium borate) may be added to prevent pH drift under storage conditions.

Ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0 percent by weight, based on the total weight of the composition (wt.%).

The route of administration (e.g., topical, parenteral or oral) and the dosage regimen will be determined by skilled clinicians, based on factors such as the exact nature of the condition being treated, the severity of the condition, the age and general physical condition of the patient, and so on.

As indicated above, use of compounds of the present invention to prevent or reduce angiogenesis in ophthalmic tissues is a particularly important aspect of the present invention. The compounds may also be used as an adjunct to ophthalmic surgery, such as by vitreal or subconjunctival injection following ophthalmic surgery. The compounds may be used for acute treatment of temporary conditions, or may be administered chronically, especially in the case of degenerative disease. The compounds may also be used prophylactically, especially prior to ocular surgery or noninvasive ophthalmic procedures, or other types of surgery.

The use of physiologically balanced irrigating solutions as pharmaceutical vehicles for the angiostic compounds is preferred when the compositions are administered intraocularly. As used herein, the term "physiologically balanced irrigating solution" means a solution which is adapted to maintain the physical structure and function of tissues during invasive or noninvasive medical procedures. This type of solution will typically contain electrolytes, such as sodium, potassium, calcium, magnesium and/or chloride; an energy source, such as dextrose; and a buffer to maintain the pH of the solution at or near physiological levels. Various solutions of this type are known (e.g., Lactated Ringers Solution). BSS® Sterile Irrigating Solution and BSS Plus® Sterile Intraocular Irrigating Solution (Alcon Laboratories, Inc., Fort Worth, Texas, USA) are examples of physiologically balanced intraocular irrigating solutions. The latter type of

solution is described in United States Patent No. 4,550,022 (Garabedian, et al.), the entire contents of which are hereby incorporated in the present specification by reference.

The specific type of formulation selected will depend on various factors, such as the compound or its salt being used, the dosage frequency, and the disease being treated. Topical aqueous solutions, suspensions, ointments, creams and gels are the preferred dosage forms for the treatment of pterygium, hyperkeratosis, and cheloid and polyp formation. Topical ophthalmic formulations are suitable for preventing glaucoma filtration bleb failure or scar formation associated with ophthalmic surgery.

In general, the doses used for the above described purposes will vary, but will be in an effective amount to inhibit or reduce neovascularization. As used herein, the term "pharmaceutically effective amount" to inhibit or reduce neovascularization, is that amount of a combination of two or more compounds of the present invention which inhibits formation of new blood vessels or reduces the number of blood vessels which are involved in the pathological condition. The compounds will normally be contained in these formulations in an amount from about 0.01 to about 10.0 weight/percent. Preferable concentrations range from about 0.1 to about 5.0 weight/percent. Thus, for topical administration, these formulations are delivered to the disease site one to six times a day, depending on the routine discretion of the skilled clinician. Systemic administration, for example, in the form of tablets or suppositories is useful for the treatment of polyp formation. Tablets containing 10-1000 mg of a compound can be taken 2-3 times per day depending on the discretion of the skilled clinician.

The compositions of the present invention are further illustrated by the following examples. The term "angiostatic compound" refers to any compound of the present invention, as described above.

Example 1

Topical combination compositions useful for controlling ocular neovascularization:

Component	wt.%
Angiostatic Compound	0.005-5.0
Angiostatic Compound	0.005-5.0
Tyloxapol	0.01-0.05
HPMC	0.5
Benzalkonium Chloride	0.01
Sodium Chloride	0.8
Edetate Disodium	0.01
NaOH/HCl	q.s. pH 7.4
Purified Water	q.s. 100 mL

Example 2

A preferred topical composition useful for controlling neovascularzation:

Component	wt.%
Compound A	1.0
Angiostatic Compound	0.005-5.0
Tyloxapol	0.01-0.05
HPMC	0.5
Benzalkonium Chloride	0.01
Sodium Chloride	0.8
Edetate Disodium	0.01
NaOH/HCl	q.s. pH 7.4
Purified Water	q.s. 100 mL

The above formulation is prepared by first placing a portion of the purified water into a beaker and heating to 90°C. The hydroxypropylmethylcellulose (HPMC) is then added to the heated water and mixed by means of vigorous vortex stirring until all of the HPMC is dispersed. The resulting mixture is then allowed to cool while undergoing mixing in order to hydrate the HPMC. The resulting solution is then sterilized by means of autoclaving in a vessel having a liquid inlet and a hydrophobic, sterile air vent filter.

The sodium chloride and the edetate disodium are then added to a second portion of the purified water and dissolved. The benzalkonium chloride is then added to the solution, and the pH of the solution is adjusted to 7.4 with 0.1M NaOH/HCl. The solution is then sterilized by means of filtration.

The angiostatic compounds are sterilized by either dry heat or ethylene oxide. If ethylene oxide sterilization is selected, aeration for at least 72 hours at 50°C. is necessary. The sterilized angiogenic compound is weighed aseptically and placed into a pressurized ballmill container. The tyloxapol, in sterilized aqueous solution form, is then added to the ballmill container. Sterilized glass balls are then added to the container and the contents of the container are milled aseptically at 225 rpm for 16 hours, or until all particles are in the range of approximately 5 microns.

Under aseptic conditions, the micronized drug suspension formed by means of the preceding step is then poured into the HPMC solution with mixing. The ballmill container and balls contained therein are then rinsed with a portion of the solution containing the sodium chloride, the edetate disodium and benzalkonium chloride. The rinse is then added aseptically to the HPMC solution. The final volume of the solution is then adjusted with purified water and, if necessary, the pH of the solution is adjusted to pH 7.4 with NaOH/HCl.

Example 3

Formulation for oral administration:

Tablet:

10-1000 mg of two angiostatic compounds with inactive ingredients such as starch, lactose and magnesium stearate can be formulated according to procedures known to those skilled in the art of tablet formulation.

Example 4

Formulation for sterile intraocular injection:

Component	each mL contains:
Angiostatic Compound	10-100 mg
Angiostatic Compound	10-100 mg
Sodium Chloride	7.14 mg
Potassium Chloride	0.38 mg
Calcium chloride dihydrate	0.154 mg
Magnesium chloride hexahydrate	0.2 mg
Dried sodium phosphate	0.42 mg
Sodium bicarbonate	2.1 mg
Dextrose	0.92 mg
Hydrochloric acid or sodium hydroxide	q.s., pH to approx. 7.2
Water for injection	q.s.

Example 5

Preferred formulation for a topical ocular solution:

Component	wt.%
Compound A	1.0%
Angiostatic Compound	0.005-5.0%
Benzalkonium chloride	0.01%
HPMC	0.5%
Sodium chloride	0.8%
Sodium phosphate	0.28%
Edetate disodium	0.01%
NaOH/HCl	q.s. pH 7.2
Purified Water	q.s. 100 mL

Example 6

A preferred formulation for oral administration:

Tablet:

5-100 mg of Compound A and 10-1000 mg of another Angiostatic Compound with inactive ingredients such as starch, lactose and magnesium stearate can be formulated according to procedures known to those skilled in the art of tablet formulation.

Example 7

Formulations for topical dermatological use:

Cream: 1 mg/g each of two angiostatic compounds in cream base of purifed water, emulsifying wax, propylene glycol, stearic acid, isopropyl palmitate, synthetic beeswax, polysorbate 60, potassium sorbate, sorbic acid, propyl gallate, citric acid, and sodium hydroxide.

Ointment: 1 mg/g each of two angiostatic compounds in base of mineral oil and polyethylene.

Example 8

Formulation for suppository:

10-500 mg each of two angiostatic compounds with the following inactive ingredients: glycerin, butylateal hydroxytoluene, butylated hydroxyanisole, edetic acid, polyethylene glycol, and sodium chloride.

Some of the compounds of the present invention may contain a nonsteroidal anti-inflammatory agent (NSAIA) component or a calcium channel blocker (flunarizine) component. These individual moieties may add additional pharmaceutical benefit to the angiostatic efficacy of compounds of formula (I).

The compounds of formula (I) are synthesized by known methods in the art. Compounds containing a non-steroidal anti-inflammatory agent (flurbiprofen or naproxen) can be made by methods illustrated in Scheme 1 and 2, and Examples 11-14.

Compounds containing a flunarizine moiety may be made by methods disclosed in commonly assigned PCT Patent Publication No. WO/9515958, the entire contents of which are hereby incorporated by reference. Other compounds of formula (I) are commercially available from: Sigma Chemical Co. (St. Louis, Missouri) and Aldrich Chemical Co. (Milwaukee, Wisconsin).

Scheme 1

$$\underset{\text{II}}{\text{A-OH}} + \underset{\text{III}}{\text{H-X-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \longrightarrow \underset{\text{I}}{\text{A-X-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \quad \text{(eq.1)}$$

$$\underset{\text{II}}{\text{A-OH}} \longrightarrow \underset{\text{IV}}{\text{A-Cl}} + \underset{\text{III}}{\text{H-X-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \longrightarrow \underset{\text{I}}{\text{A-X-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \quad \text{(eq.2)}$$

$$\underset{\text{II}}{\text{A-OH}} \longrightarrow \underset{\text{V}}{\text{A-O}^-\text{M}^+} + \underset{\substack{\text{L=Cl, BR, I, OMs, OTs} \\ \text{VI}}}{\text{L-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \longrightarrow \underset{\text{I}}{\text{A-O-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \quad \text{(eq. 3)}$$

$$\underset{\text{II}}{\text{A-OH}} \longrightarrow \underset{\text{V}}{\text{A-O}^-\text{M}^+} + \text{Br-CH}_2\text{-C(O)OEt} \longrightarrow \underset{\text{VII}}{\text{A-O-CH}_2\text{-C(O)OEt}} +$$

$$\underset{\text{VIII}}{\text{H-NR-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \longrightarrow \underset{\text{I}'}{\text{A-NR-(CH}_2)_n\text{-Y-(CH}_2)_m\text{-Z}} \quad \text{(eq. 4)}$$

The conversion of the carboxylic acid containing nonsteroidal anti-inflammatory agents (II) to esters or amides (I) may be carried out by the following methods:

(i) As illustrated in equation 1 above, carboxylic acids (II) may be reacted with the appropriate amine or alcohol derivative (III) in the presence of a coupling reagent, such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide HCl, and 4-dimethylamine pyridine or 1-hydroxybenzotriazole, in an inert organic solvent, such as acetonitrile or tetrahydrofuran, and at a temperature from 0°C to 50°C.

(ii) As illustrated in equation 2 above, carboxylic acids (II) may be converted to acid chlorides (IV) by reacting them with a reagent such as thionyl chloride or oxalyl chloride, in the presence of an inert solid or neat, at a temperature from 0°C to 80°C. The resulting acid chloride (IV) may be reacted with the desired amine or alcohol (III) in an inert solvent such as tetrahydrofuran, in the presence of pyridine or a tertiary amine, such as triethylamine.

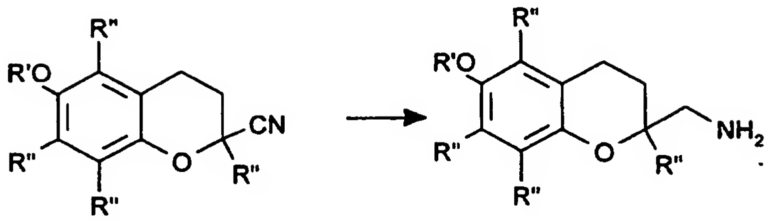
(iii) As illustrated in equation 3 above, esters (I) may be formed by reacting carboxylate anions (V), formed by reacting the carboxylic acid (II) with a base such as sodium hydride, with a halide (iodide, bromide, chloride) or sulfonate (mesylate, tosylate) (VI), in a solvent such as acetonitrile or dimethylformamide, at a temperature from 0°C to 100°C.

(iv) As illustrated in equation 4 above, amides (I) may be prepared by reacting carboxylate anions (V), formed by reacting carboxylic acid (II) with a base such as sodium hydride, with ethyl bromoacetate. The resulting ester (VII) is reacted with the desired amine (VIII), neat or in an inert solvent, such as acetonitrile or dimethylformamide, at a temperature from 0°C to 100°C.

The intermediate compounds (X) of Scheme 2 below, which can be used as compounds (III) and (VIII), were prepared using the general methods described in Cohen, et al., *Lewis Acid Mediated Nucleophilic Substitution Reactions of 2-Alkoxy-3,4-dihydro-2H-1-benzopyrans: Regiochemistry and Utility in the Synthesis of 3,4-Dihydro-2H-1-benzopyran-2-carboxylic Acids*, Journal of Organic Chemistry, volume 54, pages 3282-3292, (1989). The nitrile (IX) can be reduced using a reagent such as lithium aluminum hydride to afford the amine (X), which may be isolated as the hydrochloride salt.

The use of certain protecting groups and deprotection steps may be necessary, as will be appreciated by those skilled in the art.

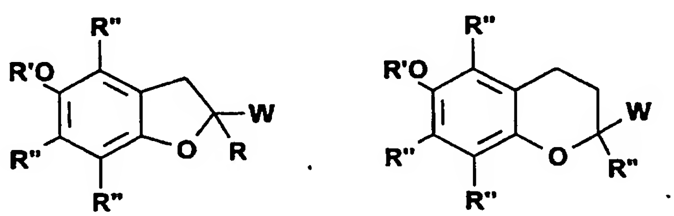
Scheme 2



IX X

Compounds of formula (I) may exist as mixtures of stereoisomers. The preparation of the individual stereoisomers may be effected by preparing and resolving the acids (II), by known methods, and then using a single stereoisomer as starting material. Compounds (III), (VI) and (VIII) may be prepared as single stereoisomers from compounds of formula (XI_{a-b}), shown in Table 5 below, using known methods:

Table 5



XI_a Xi_b

wherein:

W is $(CH_2)_p$-Q;

p is 0-1;

Q is CH_2OH or CO_2H;

R' is H, C(O)R, $C(O)NR_2$, PO_3^-, or SO_3^-; and

R" is H or C_1-C_6 alkyl.

The alcohols (XI_{a-b}) may be resolved by forming esters with optically active carboxylic acids, separating the diastereomers, and then hydrolyzing the resolved diastereomers. The corresponding carboxylic acids (XI_{a-b}) may be resolved by forming an ester with an optically active alcohol, separating the diastereomers, and then hydrolyzing the resolved diastereomers. Or, the carboxylic acids (XI_{a-b}) may be resolved by forming an amine salt with an optically active amine. Separation by recrystallization and neutralization of the resolved carboxylic acid salt may be utilized to provide the resolved carboxylic acid. Resolution of the esters and amides (I) may also be effected using chromatographic techniques known to those skilled in the art.

The amines of formula (I), where Y is NR, may be converted to amine salts by reacting the amine with acids of sufficient strength to produce an organic or inorganic salt. The pharmaceutically acceptable anions include: acetate, bromide, chloride, citrate, maleate, fumarate, mesylate, phosphate, sulfate and tartrate.

Methods of synthesizing the compounds formula (I) are further illustrated by the following examples:

Example 11

Synthesis of N-[(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzo[1,2-b]pyran-2-yl)methyl] 2-(6-methoxy-2-naphthyl)propionamide

The intermediate, (6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzo[1,2-b]pyran-2-yl)methylamine, was first synthesized:

A 1 molar (M) ethereal solution of lithium aluminum hydride (Aldrich, 32.4 mL, 32.43 mmol) was added slowly over a 5 minute period to a chilled, (4-6°C) stirring solution of (2-cyano-6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzo[1,2-b]pyran in tetrahydrofuran (50 mL). After 2 hours, the reaction mixture was quenched by the slow sequential addition of 10% aqueous tetrahydrofuran (30 mL), 15% sodium hydroxide (10 mL) and then water (20 mL), while stirring. The resulting suspension was filtered through celite, and the celite pad was washed with ethyl ether (400 mL). The organic

layer was separated, dried (Na_2SO_4), and concentrated in vacuo, resulting in a residue. A 1 M ethereal solution of hydrochloride was then added to a solution of the residue in ethyl ether (100 mL), a solid formed, and the solid was then collected by filtration and washed with ethyl ether to give 2.31 g (65.4% yield) of a white solid. The product was used crude in the next reaction.

1H-NMR (DMSO-d_6/TMS): 1.15 (s, 3H), 1.75 (t, 2H), 1.99 (s, 6H), 2.01 (s, 3H), 2.54 (t, 2H), 2.98 (s, 2H).

MS (CI): 236 (m+1).

The hydrochloride salt of (6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzo[1,2-b]pyran-2-yl)methylamine (0.30 g, 1.10 mmole) and 6-methoxy-α-methyl naphthaleneacetic acid (Aldrich, 0.28 g, 1.21 mmole) were stirred in the presence of dimethylaminopyridine (Aldrich, 0.26 g, 2.20 mmole) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Janssen Chimica-Spectrum, 0.21 g, 1.10 mmole), in tetrahydrofuran (4.0 mL) under an atmosphere of nitrogen. After stirring 17 hours at ambient temperature, the reaction mixture was diluted with ethyl acetate (70 mL), washed with water (2x 15 mL), followed by brine (15 mL) and then dried (sodium sulfate). The mixture was concentrated in vacuo and the residue subjected to flash chromatography (silica gel, 100-50:0-50, v:v, hexanes:ethyl acetate). The appropriate fractions were concentrated in vacuo, and the resulting crystalline foam suspension was then washed in hexanes to give 0.28 g (58.3% yield) of N-[(5-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-yl)methyl]-2-(6-methoxy-2-naphthyl)propionamide as a white amorphous solid.

^{1}H-NMR ($CDCl_3$) d 1.03-1.08 (d,3H), 1.57-1.64 (m, 6H), 1.70 (t, 2H,), 2.04-2.05 (m, 6H,), 2.48-2.51 (m, 2H), 3.16-3.58 (m, 2H), 3.74 (q, 1H), 3.91 (s, 3H), 4.91 (br s, 1H), 5.751 (t, 1H), 7.01-7.19 (m, 2H), 7.29-7.40 (t, 1H), 7.52-7.81 (m, 3H).

Elemental Analysis: Calculated for $C_{28}H_{33}NO_4$

Calculated: C, 75.14; H, 7.43; N, 3.13.

Found: C, 75.04; H, 7.50; N, 2.97.

Melting point: 67-70°C.

Example 12

Synthesis of 2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2yl)ethyl 2-(6-methoxy-2-naphthyl)propionate

A solution of 1,3-dicyclohexylcarbodiimide (Aldrich, 0.89 g, 4.31 mmol) in acetonitrile (25 mL), was added dropwise to a stirring slurry of (+)-6-methoxy-a-methyl-2-naphthaleneacetic acid (Aldrich, 0.90 g, 3.91 mmol), 2-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2yl)ethanol (0.98 g, 3.91 mmol, USP 5,266,709 column 45) and 1-hydroxybenzotriazole hydrate (Aldrich, 0.59 g, 4.31 mmol), in acetonitrile (50 mL). After stirring for 18 hours, the reaction mixture was concentrated in vacuo. The residue was partitioned between water (30 mL) and methylene chloride (30 mL). The layers were separated, and the aqueous layer was extracted with methylene chloride (2 x 20 mL). The combined organic extracts were washed with water (20 mL), then dried (magnesium sulfate) and concentrated in vacuo. Flash chromatography (silica gel, 2:8, v:v, ethyl acetate:hexanes) of the residue afforded a white solid upon the concentration of the appropriate fractions. The white solid was recrystallized from an ethyl acetate-hexanes mixture to give 0.60 g (33.1% yield) of 2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2yl)ethyl 2-(6-methoxy-2-naphthyl)propionate, a mixture of diastereomers, as a white solid.

^{1}H NMR ($CDCl_3$) d 1.1 (d, 3H), 1.6-1.5 (m, 3H), 1.6 (m, 2H), 1.9 (m;2H). 2.0 (s, 6H), 2.1 (s, 3H), 2.4 (t, 2H), 3.8 (q, 2H), 3.9 (s, 3H), 4.2 (s, 1H), 4.1-4.4 (m, 2H), 7.1-7.7 (m,6H).

Elemental Analysis: Calculated for $C_{29}H_{34}O_5$
Calculated: C, 75.30; H, 7.41.
Found: C, 75.24; H, 7.46.
Melting Point: 99.5-101.5°C.

Example 13

Synthesis of 2-(5-hydroxy-2,4,6,7-tetramethyl-3,4-dihydro-benzo[1,2-b]furan-2yl)ethyl 2-(6-methoxy-2-naphthyl)propionate

A solution of 2-(5-hydroxy-2,4,6,7-tetramethyl-2,3-dihydrobenzo[1,2-b]furan-2-yl)ethanol (1.30 g, 5.51 mmol) and 6-methoxy-α-methyl naphthaleneacetic acid (Aldrich, 1.39 g, 6.06 mmol) was stirred in the presence of dimethylaminopyridine (0.67 g, 5.51 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (1.06 g, 5.51 mmol), in tetrahydrofuran (25 mL). The reaction mixture was stirred at ambient temperature under nitrogen for 24 hours, diluted with ethyl acetate (150 mL), washed with water (2x 40 mL) and then brine (30 mL). The organic extract was dried (sodium sulfate) and concentrated in vacuo. The residue was subjected to flash chromatography (silica gel, 100-50:0-50, v:v, hexanes:ethyl acetate), and the appropriate fractions were combined to give 1.84 g (74.5% yield) of a foam residue. Fractional crystallization and recrystallization from methylene chloride-hexanes gave 0.40 g (13.0% yield) of white solid.

^{1}H-NMR ($CDCl_3$): 1.34 (s, 3H), 1.54-1.57 (d, 3), 1.99 (t, 2H), 2.01 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3), 2.73-2.81 (d, 1), 2.90-2.97 (d, 1), 3.77-3.89 (q, 1H), 3.91 (s, 3H), 4.102 (s, 1H, 4.165-4.29 (m, 2H), 7.10-7.16 (m, 2H), 7.35-7.40 (m, 1H), 7.64-7.70 (m, 2H).

Elemental Analysis: Calculated for $C_{28}H_{32}O_5 \cdot 0.1$ mole CH_2Cl_2.
Calculated: C, 73.84; H, 7.10.
Found: C, 73.85, 73.83; H, 7.12.
Melting point: 129.5-131°C.

Example 14

Synthesis of 2-(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo [1,2-b]pyran-2yl)ethyl 2-(3-fluoro-4-phenyl-phenyl)propionate

The intermediate, 2-(6-benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2yl)ethyl 2-(3-fluoro-4-phenyl-phenyl)propionate, was first synthesized:
A solution of flubiprofen (Sigma, 2.0 g, 8.2 mmol), 2-(6-benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo[1,2-b]pyran-2-yl)ethanol (2.4 g, 8.2 mmol) 1-hydroxybenzotriazole hydrate (Aldrich, 2.4 g, 13.9 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (Aldrich, 2.8 g, 12.3 mmol), in acetonitrile (40 ml), was stirred at ambient temperature. After 72 hours, the reaction mixture was concentrated in vacuo and the residue partitioned between water and methylene chloride. A solid formed which was removed by filtration and discarded. The layers were separated and the aqueous layer was extracted with methylene chloride (2 x 25 ml). The combined organic extracts were then dried (magnesium sulfate) and concentrated in vacuo. The residue was chromatographed (silica gel, 2:8, v:v, ethyl acetate:hexane). Concentration of the appropriate fractions afforded 3.0 g (64% yield, mixture of stereoisomers) of the product as a clear oil.

^{1}H NMR ($CDCl_3$) d: 1.23-1.27 (m, 3H), 1.53-1.57 (m, 3H), 1.75 (m, 2H), 1.95 (m, 2H), 2.08 (s, 3H), 2.14 (s, 3H), 2.21 (s, 3H), 2.55 (t, 3H), 3.75 (m, 2H), 4.3 (m, 1H), 4.65 (s, 2H), 7.1-7.7 (m, 13H).

A solution of 2-(6-benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-benzo [1,2-b]pyran-2yl)ethyl 2-(3-fluoro-4-phenyl-phenyl)propionate in ethyl acetate was treated with 10% palladium on charcoal (Aldrich, 0.5 g). The resulting mixture was hydrogenated on a Parr Apparatus [initial pressure 60 pounds/inch^2 (psi)]. After 18 hours, the reaction mixture was filtered, and the resulting solution concentrated in vacuo. The residue was subjected to flash chromatography (silica gel, 2:8, v:v, ethyl acetate:hexane). Concentration of the appropriate fractions afforded a clear oil. Hexane was added to the oil and a white solid formed upon standing. The white solid was collected by filtration to

afford 0.91 g (36% yield) of 2-6-hydroxy-2,5,7,8-tetra methyl-,4-dihydro-H-benzo[1,2-b]pyran-yl)ethyl 2-(3-fluoro-4-phenyl-phenyl) propionate as a mixture of stereoisomers.

^{1}H NMR ($CDCl_3$) d: 1.22-1.23 (m, 3H), 1.51-1.55 (m, 3H), 1.65-1.8 (m, 2H), 1.85-2.00 (m, 2H), 2.08 (s, 6H), 2.14 (s, 3H), 2.57 (t, 2H), 3.75 (q, 1H), 4.1-4.5 (m, 2H), 7.10-7.65 (m, 8H).

Elemental Analysis: Calculated for $C_{30}H_{33}FO_4$.
Calculated: C,75.60; H, 6.98.
Found: C,75.69; H,7.01.
Melting point: 85-87°C.

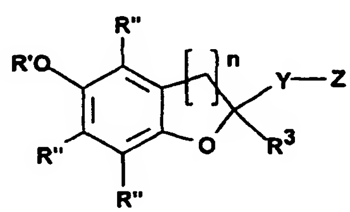
Other angiostatic compounds of the present invention are known to those skilled in the art. These compounds may be obtained by commercial sources, or synthesized by methods described in the respective publications incorporated herein or listed above.

The invention in its broader aspects is not limited to the specific details shown and described above. Departures may be made from such details within the scope of the accompanying claims without departing from the principles of the invention and without sacrificing its advantages.

What is claimed is:

1. A method of treating pathological neovascularization which comprises administering to a human a pharmaceutically effective amount of a combination of two or more angiostatic compounds.

2. A method according to Claim 1 wherein the angiostatic compounds are selected from the group consisting of: anti-mitotics, estrogen metabolites, matrix metalloproteinase inhibitors, plasminogen activator/urokinase inhibitors, urokinase receptor antagonists, platelet factor 4 and analogs, heparinases, cartilàge-derived inhibitor of angiogenesis, thrombospondin and related analogs, angiostatin, vasculostatin, proliferin-related protein, fumagillin-type compounds, tecogalan, pentosan polysulfate, thalidomide and related analogs, CM101, tyrosine kinase inhibitors, anti-sense oligonucleotides, suramin-type compounds, angiostatic steriods, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonists, cytotoxic antibodies against endothelial cell antigens, interferon, VEGF and bFGF antagonists, flk-1 and flt-1 antagonists, IL-1 and TFN antagonists, and a compound according to formula (I):



(I)

wherein:

n is 1 or 2;

R is H, C_1-C_6 alkyl or C_3-C_6 cycloalkyl;

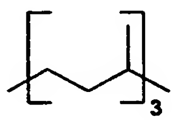
Y is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, O, NR, $C(R)_2$, CH(OH) or $S(O)_{n'}$;

n' is 0 to 2;

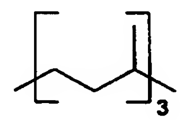
R' is H, C(O)R, $C(O)N(R)_2$, PO_3^-, SO_3^- or $HO_2C(CH_2)_2(C{=}O)$---;

R" is H or C_1-C_6 alkyl;

R^3 is H, C_1-C_6 alkyl, $(CH_2)_q(OH)$, ---$(C{=}O)O(CH_2)_qCH_3$ or



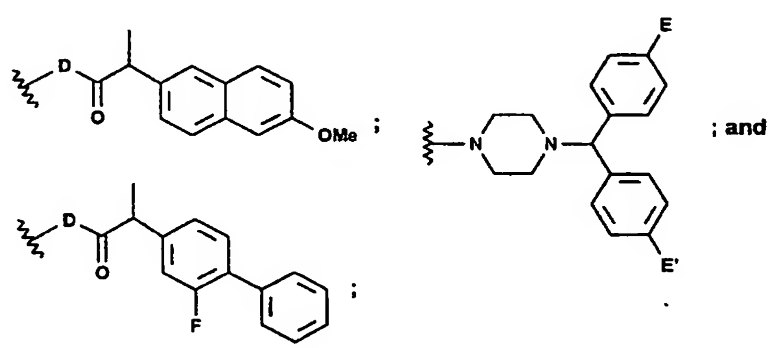
; provided that R' can not be H, when R^3 is



;

q is 1 to 10; and

Z, if present, is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, or selected from the group consisting of:



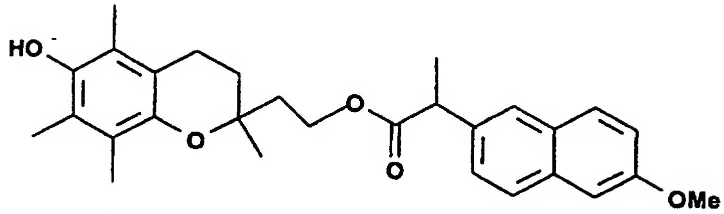
wherein:

D is O or NR; and

E and E' are independently H, F or Cl; and pharmaceutically acceptable salts thereof.

3. A method according to Claim 1, wherein: R is H, R' is H; R" is CH_3; R^3 is CH_3; and Y is C_1-C_2 alkyl.

4. A method according to Claim 1, wherein one of the compounds is selected from the group consisting of: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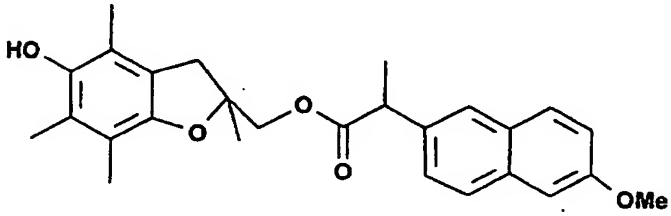
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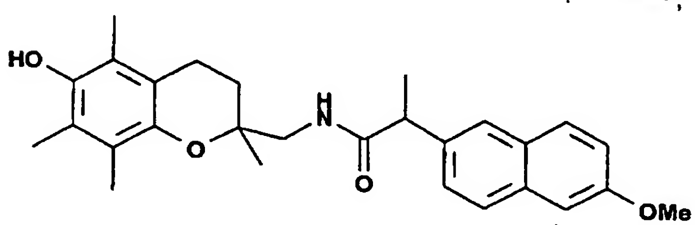
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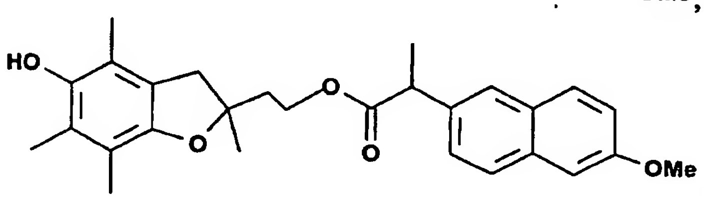
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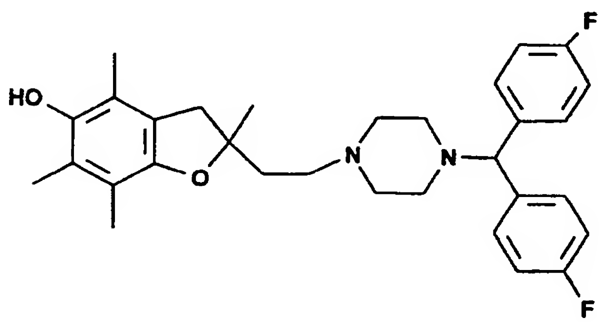
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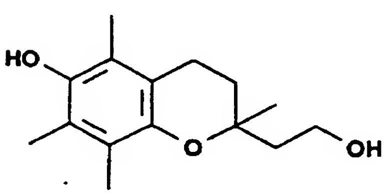
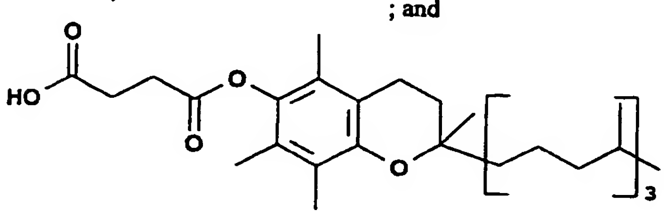

; and



5. A method according to Claim 1, wherein the compounds are combined in a single composition comprising a topical ophthalmic formulation.

6. A method according to Claim 2, wherein the compounds are combined in a single composition comprising a topical ophthalmic formulation.

7. A method according to Claim 1, wherein the compounds are combined in a single composition comprising a surgical irrigating solution.

8. A method according to Claim 2, wherein one of the compounds are combined in a single composition comprising a surgical irrigating solution.

9. A method according to Claim 1, wherein the compounds comprise an angiostatic steroid and a compound of formula (I).

10. A method according to Claim 1, wherein the compounds comprise a suramin-type compound and a compound of formula (I).

11. A method according to Claim 1, wherein the compounds comprise a fumagillin-type compound and a compound of formula (I).

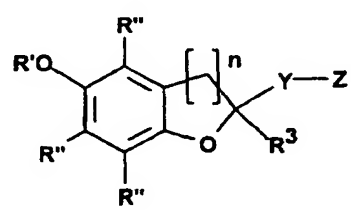
12. A method according to Claim 1, wherein the compounds comprise an angiostatic steroid and a fumagillin-type compound.

13. A method according to Claim 1, wherein the compounds comprise an angiostatic steroid and a suramin-type compound.

14. A method according to Claim 1, wherein the compounds comprise an anti-mitotic and compound of formula (I).

15. A composition for treating pathological neovascularization which comprises a pharmaceutically effective amount of a combination of two or more angiostatic compounds in a pharmaceutically acceptable vehicle.

16. A composition according to Claim 15 wherein the angiostatic compounds are selected from the group consisting of: anti-mitotics, estrogen metabolites, matrix metalloproteinase inhibitors, plasminogen activator/urokinase inhibitors, urokinase receptor antagonists, platelet factor 4 and analogs, heparinases, cartilage-derived inhibitor of angiogenesis, thrombospondin and related analogs, angiostatin, vasculostatin, proliferin-related protein, fumagillin-type compounds, tecogalan, pentosan polysulfate, thalidomide and related analogs, CM101, tyrosine kinase inhibitors, anti-sense oligonucleotides, suramin-type compounds, angiostatic steriods, $\alpha_v\beta_3$ and $\alpha_5\beta_3$ integrin antagonists, cytotoxic antibodies against endothelial cell antigens, interferon, VEGF and bFGF antagonists, flk-1 and flt-1 antagonists, IL-1 and TFN antagonists, and a compound according to formula (I):



(I)

wherein:

n is 1 or 2;

R is H, C_1-C_6 alkyl or C_3-C_6 cycloalkyl;

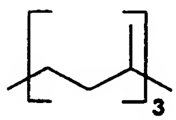
Y is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, O, NR, $C(R)_2$, CH(OH) or $S(O)_{n'}$;

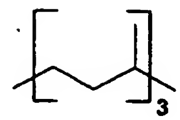
n' is 0 to 2;

R' is H, C(O)R, $C(O)N(R)_2$, PO_3^-, SO_3^- or $HO_2C(CH_2)_2(C{=}O)$---;

R" is H or C_1-C_6 alkyl;

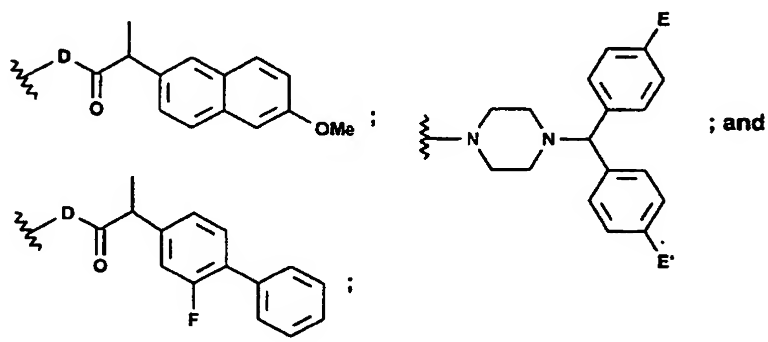
R^3 is H, C_1-C_6 alkyl, $(CH_2)_q(OH)$, ---$(C{=}O)O(CH_2)_qCH_3$ or



; provided that R' can not be H, when R^3 is  ;

q is 1 to 10; and

Z, if present, is H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, or selected from the group consisting of:



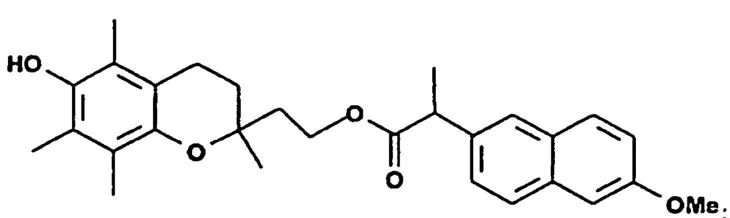
wherein:

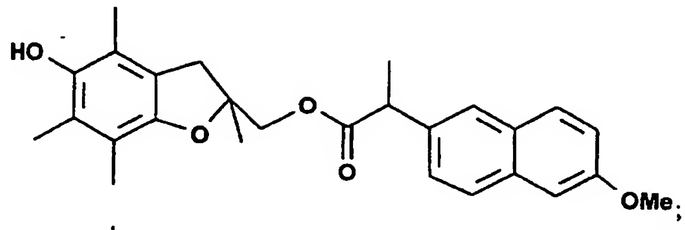
D is O or NR; and

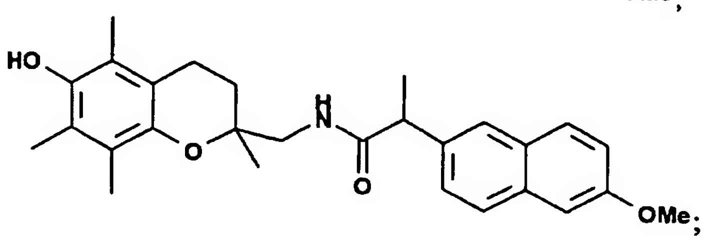
E and E' are independently H, F or Cl; and pharmaceutically acceptable salts thereof.

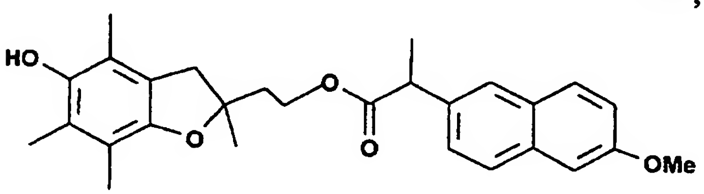
17. A composition according to Claim 15, wherein: R is H, R' is H; R" is CH_3; R^3 is CH_3; and Y is C_1-C_2 alkyl.

18. A composition according to Claim 15, wherein one of the compounds is selected from the group consisting of:









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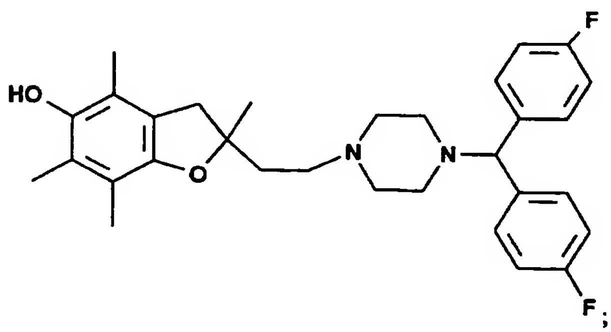
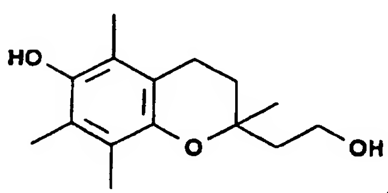
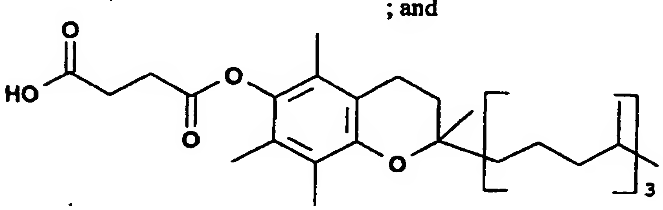




; and



19. A composition according to Claim 15, wherein the composition is a topical ophthalmic formulation.

20. A composition according to Claim 16, wherein the composition is a topical ophthalmic formulation.

21. A composition according to Claim 15, wherein the composition is a surgical irrigating solution.

22. A composition according to Claim 16, wherein the composition is a surgical irrigating solution.

23. A composition according to Claim 15, wherein the compounds comprise an angiostatic steroid and a compound of formula (I).

24. A composition according to Claim 15, wherein the compounds comprise a suramin-type compound and a compound of formula (I).

25. A composition according to Claim 15, wherein the compounds comprise a fumagillin-type compound and a compound of formula (I).

26. A composition according to Claim 15, wherein the compounds comprise an angiostatic steroid and a fumagillin-type compound.

27. A composition according to Claim 15, wherein the compounds comprise an angiostatic steroid and a suramin-type compound.

29. A composition according to Claim 15, wherein the compounds comprise an anti-mitotic and compound of formula (I).